Antigens and Antibodies

Introduction to Immunoglobulins

Even though they're technically not the same thing—and, as we'll see, circulating immunoglobulins do behave differently than membrane-bound immunoglobulins—we're teaching antibodies, immunoglobulins, and antigen receptors of B cells as the same thing. The immunoglobulin-shaped membrane-bound protein that is the surface antigen receptor on a B cell is the **exact same thing** (shape, size, function, pieces, how it's made, where it's made) as a circulating immunoglobulin-shaped antibody, just not attached to the cell membrane.

Recognize that the cells of the B cell lineage make **either** membrane-bound receptors OR secreted antibodies. B cells make immunoglobulins. **Memory (B) cells** and **mature naive B cells** make immunoglobulins that are **membrane-bound**, **surface**, **antigen receptors**. Activated B cells called **plasma cells** make immunoglobulins that are **not membrane-bound** and are secreted as antibodies. The only difference is that one is membrane-bound (immunoglobulins from memory and mature naive B cells) and the other (antibody from plasma cells) is not.

Those immunoglobulins that are secreted as antibodies can interact with other antibodies in circulation, polymerizing with each other. Being surface-bound, the immunoglobulin is already membrane-bound, so it can't polymerize. Some immunoglobulins polymerize when they are secreted. We use **monomer** to mean a complete, two-halved Y-shaped immunoglobulin. We use **monomer-half** to refer to the half of a Y-shaped immunoglobulin consisting of a single heavy chain and single light chain. Monomer-half is my word I use to avoid confusion between the maturation of a B cell and the function of antibodies in circulation.

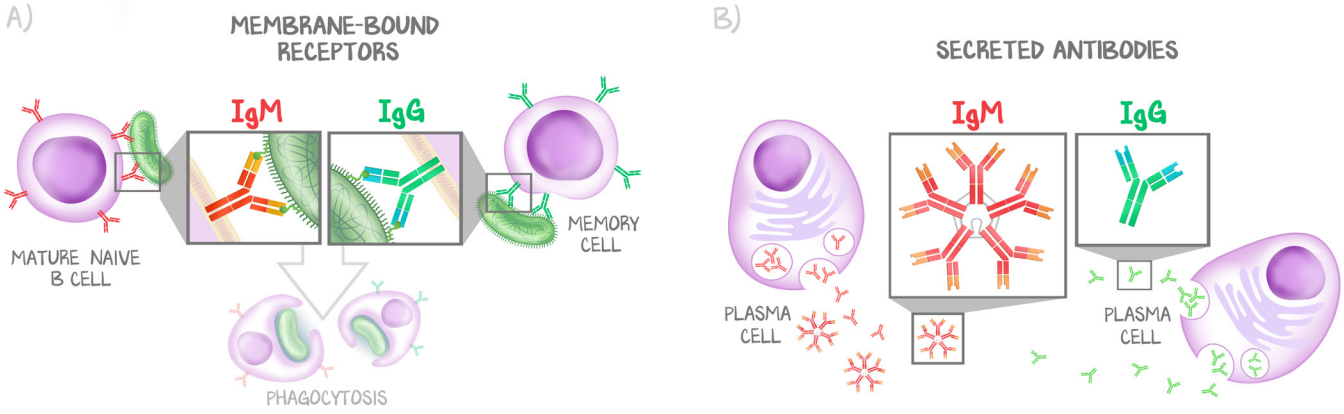

Figure 6.1: The Various Roles of Immunoglobulins
(a) Mature naive B cells and memory cells have the immunoglobulin-shaped structure attached to the plasma membrane. Here, the immunoglobulin acts as a receptor; when it binds antigen, it activates a cytoplasmic signal (to induce phagocytosis). (b) Plasma cells have the immunoglobulin-shaped structure in their cytoplasm, within vesicles, not attached to membranes. Here, the immunoglobulin-shaped structure is an antibody, which will be excreted by the plasma cell to circulate in the bloodstream.

Antibodies bind to **antigen**. Immunoglobulins are antibodies. Therefore, immunoglobulins bind to antigen. Immunoglobulins as surface proteins on B cells are membrane-bound. They still bind to antigen. Immunoglobulins secreted as antibodies from B cells (that may or may not polymerize in circulation) bind antigen. This binding is **very specific** to the antigen, and in the process of B-cell maturation and activation, gets **even more hyperspecific** to that one antigen.

The repetition of these similarities and differences in this introduction section was deliberate. If this concept was easy for you, congratulations! Most people have trouble with it. If you're most people, swallow the pride, and read the introduction again unless you can describe out loud, without looking, everything discussed above. This section is so long because I repeat the core concepts over and over again.

Structure of Immunoglobulins

The smallest unit of an immunoglobulin is a **two-chain structure** that I call a **monomer-half**. The **monomer immunoglobulin** is the combination of **two monomer-halves** to form a **four-chain structure**. Each monomer-half is identical to the other. Each monomer-half consists of a longer **heavy chain** (four domains long) and a shorter **light chain** (two domains long).

The first domain of every chain, whether light or heavy, is variable, and the rest are constant. Because the light chain is only two domains long, the first is a **variable domain** and the second is a **constant domain**. Because the heavy chains are four domains long, the first is a **variable domain**, and the rest are **three constant domains**. A light chain and a heavy chain line up so that each variable domain is aligned with its corresponding variable domain on the opposing chain. The variable region, the combination of both variable domains, identifies antigen. The constant domains are how the chains stay connected. The light chain is attached to the heavy chain by **disulfide bonds**. Just below the first constant domain, the chains are connected by a disulfide bond. When the two monomer-halves come together, they're also connected by **disulfide bonds**. The connection between the two heavy chains of each monomer-half is made between the first and second constant domains. This creates the **hinge region**. The heavy chains form the bottom of the Y structure, and light and heavy chains form the tips of the Y structure.

The bottom of the Y is made up entirely of heavy-chain constant domains. This is the **Fc** portion of the immunoglobulin. It's named Fc because it's the fragment that represents the **cytoplasmic** end of the immunoglobulin when it acts as a receptor. Its name comes from what happens when we take a normal immunoglobulin and expose it to a chemical, pepsin. When exposed to pepsin, the free fragment is the Fc fragment. This portion determines the **isotype** of the immunoglobulin, and is responsible for the immunoglobulin's behavior when secreted as an antibody. Different isotypes are IgM, IgG, IgA, and IgE. More on this later.

The tips of the Y are made of both heavy and light chains, both of which are made of one constant domain and one variable domain. It's the **variable domains that matter** when it comes to antigen recognition and binding. These variable domains comprise the **antigen-binding site**, and it's called **Fab** for the fragment that is the antigen-binding site when exposed to **papain** ("a" for both the "a" in papain and in Fab). Whereas the isotype was determined by the Fc portion, the **idiotype** is defined by the Fab. Idiotype means what specific antigen the immunoglobulin will bind to. Within the variable region there's also a **hypervariable region** where the variability is significantly higher. This is also referred to as a **complementarity-determining region**. This antigen-binding site is actually a sheath, or a sleeve, and this sleeve can change shape to best fit a specific antigen, meaning it can best identify and bind a specific antigen.

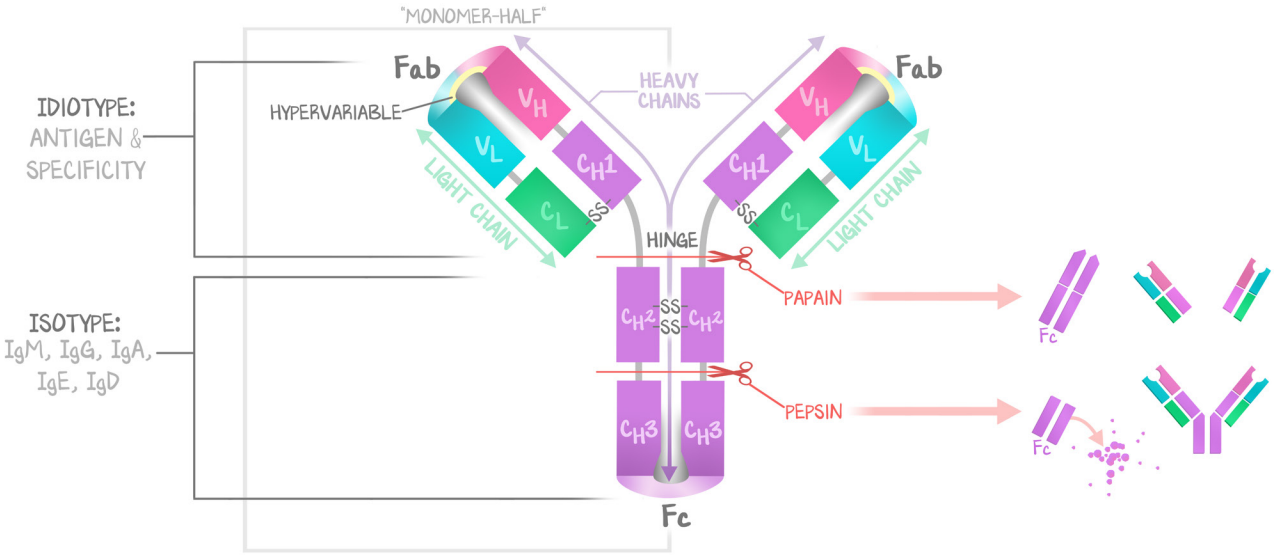

Figure 6.2: Structure of Immunoglobulins
This is a schematic of an immunoglobulin, demonstrating the four domains of the heavy chain and the two domains of the light chain, and how they functionally connect with disulfide bonds. A more accurate representation of the how the chains pair, creating the hinge region. What fragments look like when exposed to the chemicals papain (revealing the Fab) and pepsin (revealing the Fc).

Affinity and Avidity

Affinity is the intrinsic binding strength of one **antigen-binding site** on one immunoglobulin. The more an Fab portion wants to bind a specific antigen, the higher the affinity it has for that Fab portion. A B-cell surface receptor immunoglobulin can have an affinity, and a circulating antibody can have an affinity.

Avidity is the **sum total** binding strength of all binding sites in a molecule. It refers to **circulating antibodies only**. An immunoglobulin acting as a membrane-bound surface protein can't polymerize, and therefore can't combine the affinities of multiple immunoglobulins into a sum total. If the circulating antibody exists as a monomer (such as IgG), then its avidity is the affinity. But certain circulating antibodies can form polymers. The polymer would have an avidity equal to the product of the affinity of one molecule multiplied by the number of molecules. For example, IgM forms a **pentamer when circulating**. We'll use fabricated numbers to illustrate the point.

	IgM	IgG
Polymeric Form	Pentamer (5)	Monomer (1)
Affinity	1	20
Avidity	5 × 1 = 5	1 × 20 = 20

Table 6.1: Affinity and Avidity

The point is that IgGs exist as monomers because their affinity for their antigen is so high that they need only a monomeric form to function. But also realize that IgG has an extraordinarily low affinity for any other antigen other than the one it is meant to bind to. In comparison, IgM is nonspecific, and increases its function by existing as a pentamer, increasing avidity. It won't reach the avidity of IgG, but because it is less specific, it can also bind to more antigens than the IgG can. It's not surprising, then,

that IgM is the default antibody, and IgG is the specialized form. Clinically this may be important, as we want IgM to recognize as many antigens (foreign invaders) as possible. Once a foreign invader is recognized, the immune system creates a more specific IgG to help recognize it and eliminate it not only at the present time, but in case it invades again in the future. Having a highly specific IgG with high affinity will help the antibody find and bind to that antigen, should it ever see it again.

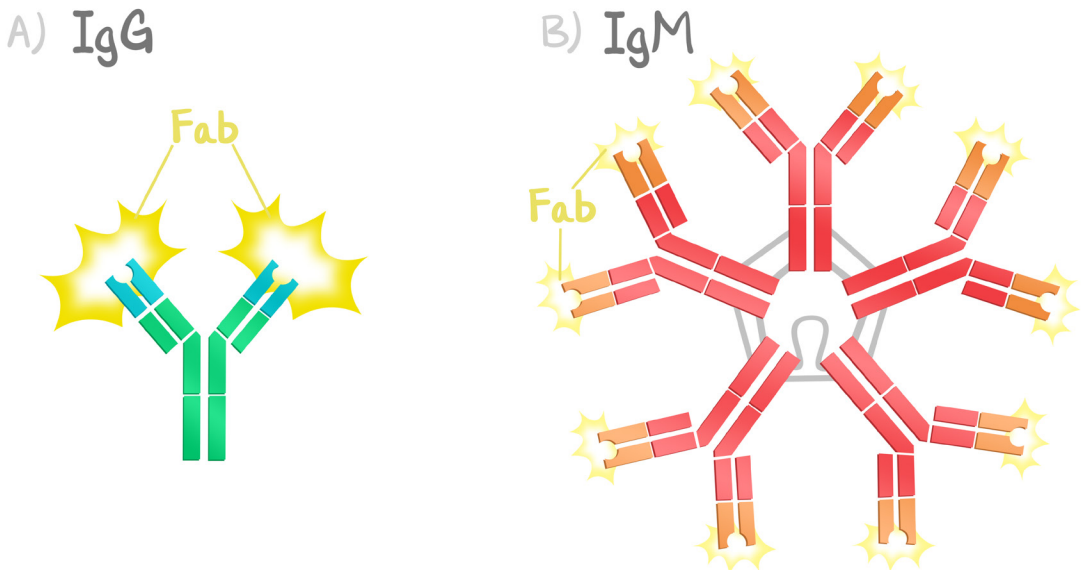

Figure 6.3: Affinity and Avidity
The cell-membraned protein is a monomer. IgG is a monomer. IgM can form a pentamer. It forms the pentamer so that it can bend all five of its monomers to a single antigen. (a) Monomer of IgG with high affinity. (b) Pentamer of IgM combining low affinities together to muster avidity. (c) Electron microscopy demonstrating the pentamer and the bending of all immunoglobulins to the antigen.

Immunoglobulins as Antibodies (Antibody Function)

Antibodies are circulating immunoglobulins. Antibodies have an affinity, an avidity, an antigen-binding site, and an Fc portion. When released into circulation, antibodies bind to their antigen using their antigen-binding site. When an antibody gets hold of an antigen, several things can happen: neutralization, opsonization, and complement activation.

Neutralization is the act of the antibodies being a **physical barrier** between membrane proteins on pathogens and membrane proteins on cells. Coating a pathogen in antibodies blocks the effect of pathogen cell-membrane proteins, rendering them useless. It also prevents collision with other cells, which is especially important to prevent viral entry into cells.

Opsonization makes **phagocytosis easier**. Antibodies make the pathogen unable to interact with host cells, except for phagocytes. Phagocytes, especially macrophages, have an Fc receptor. The Fab portion is the antigen-binding site, attached to the antigen. The same Fc portion that helped the immunoglobulin stay connected to the B-cell membrane as a membrane-bound surface protein helps the immunoglobulin stay connected to the phagocyte membrane. This lets the phagocyte grab hold of whatever it is trying to phagocytose, making it less likely for the pathogen to escape, thereby improving phagocytosis.

Phagocytes aren't the only thing that can bind Fc. The **complement cascade** is initiated by the Fc portion of the immunoglobulin. This activation of complement using antibodies is deemed the **classical pathway**, starting with C1 and moving into the MAC attack complex, C5b–9, discussed below.

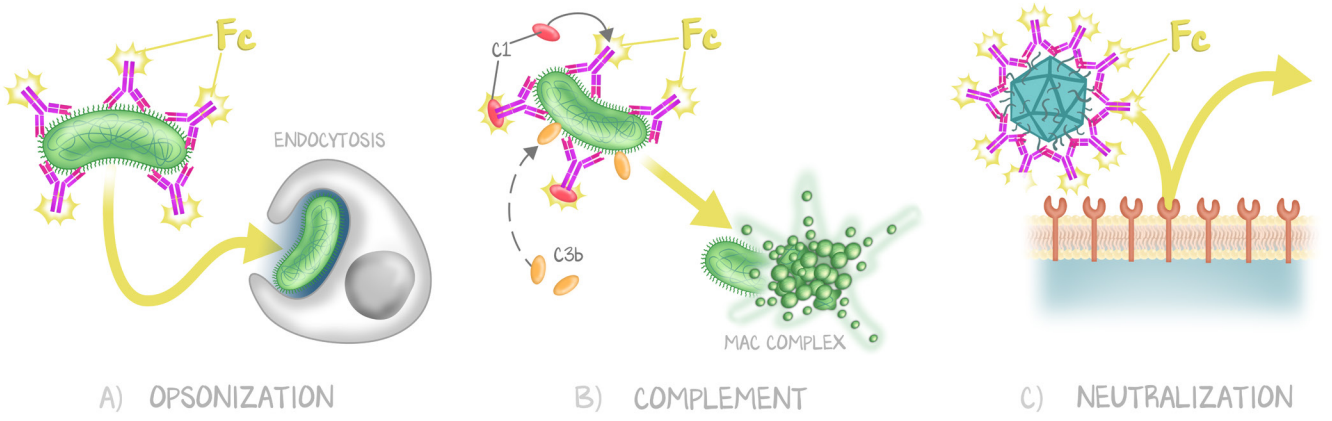

Figure 6.4: Purpose of Antibodies
When the Fab portion binds antigen, it binds and holds on to the thing with the antigen. The free Fc portion, now no longer membrane-bound, can bind other things. (a) Fc receptors on phagocytes increase phagocytosis. (b) Fc is the site for complement (especially C1) to stick to. (c) Fc forms a physical barrier for particles that require physical contact to act, such as viral capsids.

Antigens and Immunogens

An **antigen** is a foreign substance capable of binding with a lymphocyte. It doesn't necessarily need to produce an immune response. Likewise, **antigenicity** is the ability to react with an antibody-combining site. It doesn't mean that if bound something happens, only that it could bind. In sufficient doses, an antigen would become an immunogen, provoking an immune response. All pieces of every cell are antigens.

An **immunogen** is an antigen that WILL bind a lymphocyte. Likewise, **immunogenicity** is the ability to actually induce an immune response. Pieces of cells that induce immune responses are **immunogens**.

Different Structures of Different Antibodies

All immunoglobulins have two Fab portions. All immunoglobulins have one Fc portion. All **membrane-bound surface-protein immunoglobulins** exist as monomers tethered to the cell membrane, with the Fab portion exposed to the extracellular space and the Fc portion bound to the cell membrane. **Circulating immunoglobulins** are antibodies, and circulating immunoglobulins can take on polymeric forms, connecting their Fc portions together with a **J chain** at their center, with the Fc portion as the stick of the Y. At the very end of the Fc portion is the binding site that does the business—opsonization, neutralization, and complement activation. The J chain connects antibodies

together by the Fc portion, but it connects at a different physical location than the business spot. The Fc portions remain accessible, so their functioning is preserved, but the J chain holds the polymer together.

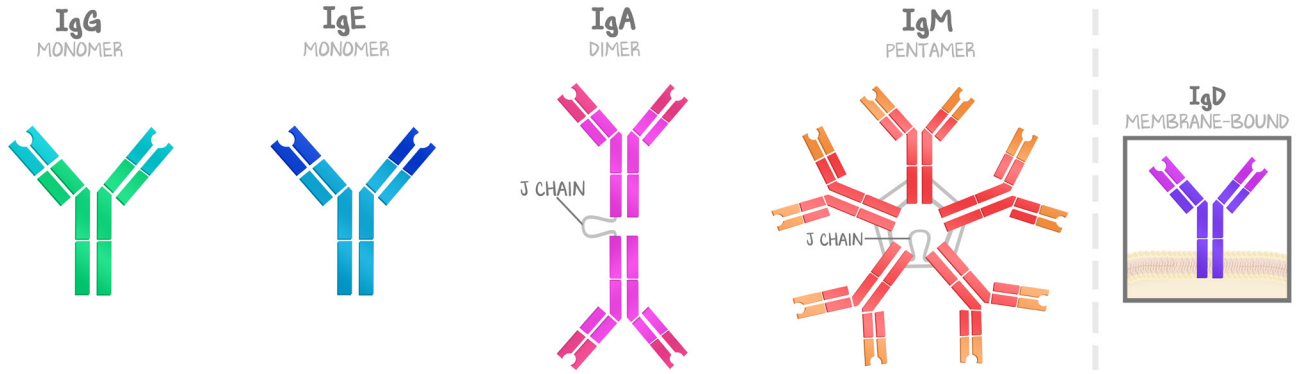

Figure 6.5: Immunoglobulins Can Polymerize
Based on the isotype, determined by the Fc region, immunoglobulins secreted as antibodies can form various polymers. All IgM behaves the same way, all IgA, etc.

IgG is a monomer. **IgE** is a monomer. **IgA** exists as a **dimer** and is found in the **mucosal surfaces** (gut and lungs). **IgM** is a **pentamer**. IgD is the immunoglobulin of impotence, is only ever membrane-bound, is never secreted as an antibody, and is discussed in greater detail in #7: *B-Cell Maturation*.

The membrane-bound immunoglobulins of naive B cells and memory cells are monomers because they are membrane-bound. Remember, membrane-bound immunoglobulins cannot polymerize. The secreted immunoglobulins of activated B cells and plasma cells can be polymeric, depending on the isotype.

The Complement Cascade

The complement cascade starts with C1 and finishes with C9. One through nine. Sounds simple, right? Wrong. DO NOT MEMORIZE THE CASCADE. Only remember the specific complements that we mention here.

In the **classical pathway**, antibodies bind to their target antigen. Just as immunoglobulins facilitate phagocytosis of pathogens using their Fc portion, so too do immunoglobulins make it easier for C1 to bind the pathogen by binding to Fc. Since the start of the complement cascade is more readily available at the site of antibody adhesion, the entire cascade is likely to get on its way at the same spot. From C1 the cascade happens. Through the process, complements are cleaved into their "a" portion and their "b" portion (C3 → C3a and C3b). **C3b** acts just like an antibody. It sticks to a pathogen and makes phagocytosis easier. It also lets the rest of the complement cascade continue. **C5b** starts the **MAC attack complex (5–9)**, which then creates a hole in the cell membrane of the target cell. The cell being without its lipid bilayer, that hole creates a loss of electric charge across the plasma membrane; the cell depolarizes, and dissolves through the hole. Other factors help in the immune response in other ways. For example, C3a, C4a, and C5a are all involved in chemotaxis and the recruitment of white cells. The **"a's" do chemotaxis**.

The **alternate pathway** does not require an antibody to be bound to the pathogen. That means it does not start with C1. It starts with C3b. Yes, the same C3b from the last paragraph. C3b is created with activation of the classical pathway, starting with C1. But C3b is also circulating in the blood. That circulating C3b can attach to a pathogen even without the Fc portion of an immunoglobulin being present. C3b does the same thing it did in the classical pathway. The difference, the reason it is "alternate," is because C3b attached to the pathogen without an antibody and began the cascade at an "alternate" location in the pathway.

The **mannan-binding lectin** pathway (also known as the mannose-binding lectin pathway) also activates the complement cascade, but does not recognize antibodies. Instead, certain organisms happen to have the correct terminal sugars on their glycocalyx. Mannose-binding lectin bumps into these sugars, and acts as the site of complement activation. This is akin to the way phagocytes have nonspecific receptors that recognize foreign particles.